

TRYPTOPHAN METABOLISM AS RELATED TO THE B VITAMINS

BY

IRWIN MORTON WEINSTOCK

B.S., University of Oklahoma, 1947

M.S., University of Illinois, 1948

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
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I. THE EFFECT OF RIBOFLAVIN, VITAMIN B₆, AND THIAMINE DEFICIENCIES ON THE METABOLISM OF TRYPTOPHAN BY THE RAT

INTRODUCTION

Several workers have reported that a vitamin B₆ deficiency reduces the conversion of administered tryptophan to urinary N¹-methylnicotinamide (1,2) and to blood pyridine nucleotides (3). It has also been reported that riboflavin and thiamine deficiencies reduce the conversion of tryptophan to urinary N¹-methylnicotinamide (4).

Quinolinic acid accounts for a greater per centage of administered tryptophan and 3-hydroxyanthranilic acid than does any other known pyridine derivative in the urine (5,6). Excretion of this compound was measured after administration of L-tryptophan, DL-kynurenine sulfate, and 3-hydroxyanthranilic acid in an effort to locate more definitely the site of vitamin action and to obtain further evidence of the importance of certain vitamins in the conversion of tryptophan to nicotinic acid and related compounds.

EXPERIMENTAL

Rats were fed a 9% casein diet with the omission of single vitamins until they became deficient. The test compounds (0.25 mM) were injected intraperitoneally into control and deficient animals. Control animals excreted $9.83 \pm .91\%$ of the administered L-tryptophan, $0.78 \pm .07\%$ of the DL-kynurenine sulfate, and $11.31 \pm 1.37\%$ of the 3-hydroxyanthranilic acid as quinolinic acid. Riboflavin deficiency reduced the amount of administered L-tryptophan appearing as quinolinic acid to $1.00 \pm .07\%$ and the amount of DL-kynurenine sulfate appearing as this urinary product to $0.34 \pm .05\%$. During vitamin B₆ deficiency $1.14 \pm .30\%$ of the administered L-tryptophan and $0.325 \pm .063\%$ of the administered DL-kynurenine sulfate were excreted as urinary quinolinic acid.

Inducing a more severe vitamin B₆ deficiency by incorporating desoxypyridoxine in the diet caused a further reduction in the amounts of tryptophan and kynurenine appearing as quinolinic acid. Quinolinic acid excretion in response to 3-hydroxyanthranilic acid administration was not affected by either a riboflavin or vitamin B₆ deficiency. The reduced excretion of quinolinic acid by riboflavin and vitamin B₆-deficient rats in response to tryptophan and kynurenine, but not to 3-hydroxyanthranilic acid, indicates that these vitamins function in the conversion of kynurenine to 3-hydroxyanthranilic acid.

Thiamine deficiency reduced the excretion of quinolinic acid following both tryptophan and 3-hydroxyanthranilic acid administration, but the effect appeared to be due to inanition. Results obtained with paper strip chromatography indicated that quinolinic acid was the only product being measured.

SUMMARY

Riboflavin and vitamin B₆-deficient rats excreted much smaller amounts of quinolinic acid following tryptophan and kynurenine administration than did control animals. These deficiencies did not affect quinolinic acid excretion after 3-hydroxyanthranilic acid administration. These results strongly suggest that these vitamins function in the conversion of kynurenine to 3-hydroxyanthranilic acid.

II. THE TRYPTOPHAN-NICOTINIC ACID RELATIONSHIP IN MICROORGANISMS

INTRODUCTION

The relationship of tryptophan, nicotinic acid, and vitamin B₆ in mammals has been reported in the previous section

(Chap. I.) and by other workers (1-4). It seemed worthwhile to investigate this relationship in microorganisms as well as to study the ability of tryptophan, kynurenine, 3-hydroxyanthranilic acid, and quinolinic acid to replace the requirement of various microorganisms for nicotinic acid.

EXPERIMENTAL

Nicotinic acid concentrations as high as 1 mg. per 10 ml. of media were used with varying limited levels of vitamin B₆ to determine whether or not nicotinic acid could exert a sparing effect on the vitamin B₆ requirement of several yeasts. The growth of the vitamin B₆-requiring yeasts, *Saccharomyces carlsbergensis* ATCC 4228, *Saccharomyces oviformis* (unknown strain), and *Saccharomyces chodati* Y1536, was slightly greater when nicotinic acid was added to the media in the absence of added vitamin B₆. In the presence of vitamin B₆, added nicotinic acid had no effect. With *Kloeckera brevis* Y915, which requires both preformed nicotinic acid and vitamin B₆, the addition of either vitamin in the absence of the other resulted in a slight stimulation of growth. Though vitamin B₆ may be involved in the synthesis of nicotinic acid by these yeasts, growth of these organisms in the absence of vitamin B₆ is more seriously restricted by the failure of other processes involving this vitamin.

With none of the microorganisms investigated did L-tryptophan, DL-kynurenine sulfate, or 3-hydroxyanthranilic acid have any ability to replace nicotinic acid for growth. Quinolinic acid had a low order of growth promoting activity for all of the organisms tested. This activity, as compared to the effectiveness of nicotinic acid on a molar basis, was 0.0038% for *Lactobacillus arabinosus* 17-5, 0.0076% for *K. brevis* Y915, 0.0059% for *Torula cremoris* (unknown strain), 0.0068% for *Schizosaccharomyces pombe* 2476, 0.0058% for *Saccharomyces ludwigii* Y970, 0.004% for *Proteus* OX19, and 0.0055% for *Proteus vulgaris* 211. The variation in the growth promoting activity of quinolinic acid for these organisms suggests that this activity is not due to

contamination of the quinolinic acid used. The addition of Tween 80 and riboflavin, and lowering the pH of the media, in an effort to affect permeability, had no effect on the utilization of any of the compounds tested.

SUMMARY

The addition of nicotinic acid to vitamin B₆-deficient media resulted in a slight stimulation of the growth of four vitamin B₆-requiring yeasts. In the presence of suboptimum quantities of vitamin B₆, nicotinic acid had no effect on growth.

Quinolinic acid had a low order of growth promoting activity for all of the nicotinic acid-requiring organisms investigated. L-Tryptophan, DL-kynurenine sulfate, and 3-hydroxyanthranilic acid were completely ineffective in replacing this vitamin.

III. THE CONVERSION OF TRYPTOPHAN TO NICOTINIC ACID IN VITRO

INTRODUCTION

Reports of the *in vitro* conversion of tryptophan to nicotinic acid are conflicting (7-11), but the rapid conversion of 3-hydroxyanthranilic acid to quinolinic acid seems well established (9-11). Since the latter conversion involves the formation of a pyridine nucleus from a benzene nucleus, it would seem likely that more than one step is involved in the reaction. 3,4-Dihydroxyanthranilic acid would seem to be a logical intermediate in this conversion in view of the postulated formation of an ortho dihydroxy compound preceding ring cleavage in the oxidation of aromatic compounds by certain bacteria (12). However, this compound is not active for *Neurospora crassa* (13).

EXPERIMENTAL

L-Tryptophan, DL-kynurenine sulfate, and 3,4-dihydroxyanthranilic acid were not converted to nicotinic acid or quinolinic acid after incubation for 24 hours with rat liver slices. 3-Hydroxyanthranilic acid was nearly completely converted to quinolinic acid within 30 minutes, but further incubation of quinolinic acid did not lead to the formation of nicotinic acid.

The enzyme system which catalyzes the conversion of 3-hydroxyanthranilic acid to quinolinic acid was present in high concentrations in pig and sheep livers as well as in rat liver. No adaptive increase in the concentration of the enzyme system was obtained by requiring rats to utilize tryptophan as a source of nicotinic acid or by feeding massive doses of tryptophan. Acetone-dried powders of rat liver proved to be a stable source of the enzyme system and no easily dialyzable cofactors were required. The activity was contained in the protein fraction precipitated between 40 and 60% saturation with ammonium sulfate.

By periodically removing samples from the incubation mixture and examining the protein-free filtrates of these samples spectrophotometrically, evidence was obtained for the formation of an intermediate in the conversion of 3-hydroxyanthranilic acid to quinolinic acid. The extinction increased during the first 8 minutes of the reaction and then decreased as the reaction was allowed to continue. At 0 time a typical 3-hydroxyanthranilic acid absorption spectrum, with a maximum at $298\text{ m}\mu$ was obtained. During the course of the reaction the peak shifted to $305\text{-}310\text{ m}\mu$. By the end of 30 minutes a secondary peak had developed at $268\text{ m}\mu$ which is the characteristic absorption maximum of quinolinic acid (5). Microbiological and fluorometric determination of quinolinic acid and 3-hydroxyanthranilic acid, respectively, could be correlated with the spectrophotometric data.

SUMMARY

Tryptophan, kynurenine, and 3,4-dihydroxyanthranilic

acid were not converted to quinolinic acid or nicotinic acid *in vitro* by rat liver preparations. 3-Hydroxyanthranilic acid was converted to quinolinic acid, but conversion of quinolinic acid to nicotinic acid was not observed. The characteristics of the enzyme system catalyzing the formation of quinolinic acid from 3-hydroxyanthranilic acid were studied and spectrophotometric evidence for the formation of an intermediate compound has been presented.

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VITA

Irwin Morton Weinstock was born on July 17, 1925 in New York City, where he attended elementary and high school. He entered the City College in 1941. From 1943 to 1945 he served in the Army of the United States in the American and European Theatres. He entered the University of Oklahoma in 1945 and received the Bachelor of Science degree from that institution in 1947. In 1947 he entered the Graduate College of the University of Illinois and received the Master of Science degree in Physiology in 1948. He held a Research Assistantship from September 1949 to June 1951.

